LESSON 21:
PROTOPLAST FUSION AND SOMATIC HYBRIDIZATION

Introduction

A number of strategies have been used to induce fusion between protoplasts of different strains/species. Of these, the following three (Fig.1.) have been relatively more successful. Protoplasts of desired strains/species are mixed in almost equal proportion. Generally, they are mixed while still suspended in the enzyme mixture. The protoplast mixture is then subjected to a high pH (l0.5) and high Ca^{2+} concentration (50 m $molL^{-l}$) at 37°C for about 30 min (high pH- high Ca2+ treatment). This technique is quite suitable for some species, while for some others it may be toxic.

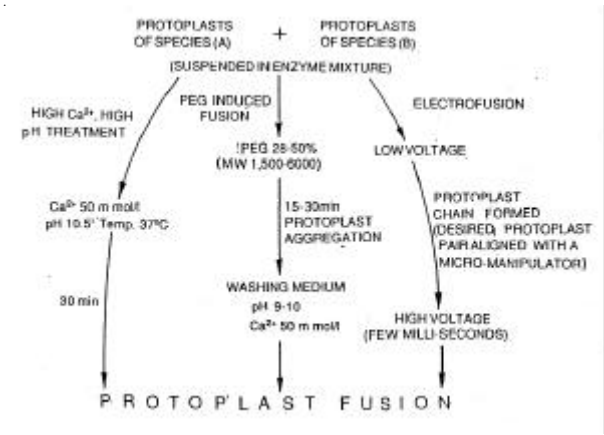

Fig. 1. A schematic representation of the 3 most successful protoplast fusion strategies.

The fact that isolated protoplasts are devoid of walls makes them easy tools for undergoing fusions in vitro. An important aspect has been that incompatibility barriers do not exist during the cell fusion process at interspecific, intergeneric, or even interkingdom levels (Ahkong et al 1975). Thus, plant protoplasts represent the finest single cell system that could offer exciting possibilities in the fields of somatic cell genetics and crop improvement.

Spontaneous Fusion

During the enzymatic degradation of cell walls some of the adjacent protoplasts may fuse together to form homokaryons (homokaryocytes). These plurinucleate cells sometimes contain 2-40 nuclei, a phenomenon attributed to expansion and subsequent coalescence of the plasmodesmatal connections between the cells. More frequent homokaryon formation has been observed in protoplasts isolated from dividing cultured cells. However, the sequential method of protoplast isolation or exposure of the cells to a strong plasmolyticum would sever the plasmodesmatal connections and, consequently, reduce the frequency of spontaneous fusion.

Mechanical Fusion

The giant protoplasts of Acetabularia have been fused mechanically by pushing together two protoplasts. This fusion does not depend upon the presence of fusion-inducing agents. However, in this procedure protoplasts are likely to get injury. Protoplasts released from meiocytes (Lilium and Trillium) in enzyme solutions readily fuse by gentle tapping in a depression slide and some of the di- and trinucleate cells reportedly complete the second division, forming tetrad configurations in culture media (Ito and Maeda 1974).

Induced Fusion

Freshly isolated protoplasts can be induced to undergo fusion, irrespective of their origin, with the help of a range of fusogens (fusion inducing agents) e.g., $NaN0_3$, artificial sea-water, lysozyme, high pH/Ca++, polyethylene glycol (PEG), antibodies, Concavalin A, polyvinyl alcohol, electrofusion, dextran and dextran sulphate, fatty acids and esters. Of these, the following treatments have yielded success in producing somatic hybrid plants.

I $NaN0_3$ Treatment

Induced fusion by $NaN0_3$ was first demonstrated by power et al. (1970). Isolated protoplasts were cleaned by floating in sucrose osmoticum. Transfer of the protoplasts in 0.25 M $NaN0_3$ solution and subsequent centrifugation promoted the fusion process. Carlson et al. (1972) used this method for producing the first somatic hybrid plant by fusing protoplasts of Nicotiana glauca and N. langsdorffii. This procedure results in a low frequency of heterokaryon formation and protoplasts are markedly altered in their uptake capabilities.

II High pH/ Ca++ Treatment

This method was developed by Kelier and Melchers (1973) for fusing two different lines of tobacco protoplasts and is now commonly used. Isolated protoplasts are incubated in a solution of 0.4 M mannitol containing 0.05 M $CaCl_2$, with pH at 10.5 (0.05 M glycine-NaOH buffer) and temperature 37°C. Aggregation of protoplasts generally takes place at once and fusion occurs within 10 min. Many intraspecific and interspecific somatic hybrids have been produced using this procedure.

III Peg Treatment

PEG has been used as a fusogen in a number of plant species because of the reproducible high frequency of heterokaryon formation accompanied with low toxicity to most cell types. About 0.6 ml of PEG solution (dissolve 1g of PEG, mol. wt. 1500 in 2 ml of 0.1 M glucose, 10 mM $CaCI_2$, and 0.7 mM KH_2P0_4) is added in drops to a pellet of protoplasts in the tube. After having capped the tube, protoplasts in PEG are incubated at room temperature for 40 min. Occasional rocking of tubes helps to bring the protoplasts in contact. This is followed by elution of PEG by the addition of 0.5-1 ml of protoplast culture medium in the tube after every 10 min.

Preparations are now washed free of fusogen by centrifugation and the protoplasts resuspended in the culture medium. Protoplast fusion occurs during the washing. The washing medium may be alkaline (pH 9-10) and contain a high Ca^{2+} ion concentration (50 m mol l^{-1}). This approach is a combination of PEG and high pH- high Ca^{2+} treatments, and is usually more effective than either treatment alone. PEG is negatively charged and may bind to cation like Ca^{2+} which, in turn, may bind to the negatively charged molecules present in plasmalemma. They can also bind to cationic molecules of plasma membrane. During the washing process, PEG molecules may pull out the plasmalemma components bound to them. This would disturb plasmalemma organisation and may lead to the fusion of protoplasts located close to each other (Fig.2.).

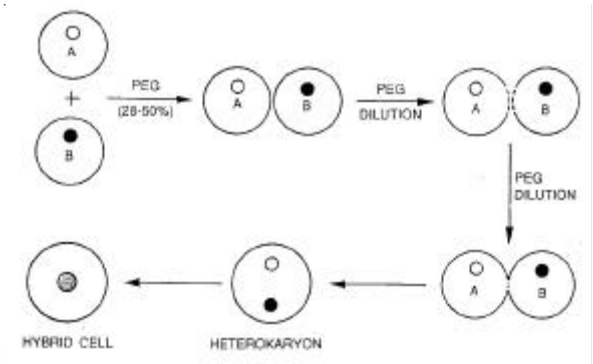

Fig.2. PEG induced protoplast fusion. Protoplasts are first brought close together (aggregation) by PEG. Fusion occurs during PEG dilution due to disturbances created in plasma membrane.

Both the mol. wt. and the concentration of PEG are critical in inducing successful fusions. PEG less than 100 mol. wt. is not able to produce tight adhesions while that ranging up to 6000 mol. wt. can be more effective per mole in inducing fusions. At higher mol. wt., however, PEG produces too viscous a solution for easy handling. After treatment with fusogen, protoplasts are cultured following the standard procedures.

IV Electrofusion

The above fusion techniques are nonselective in that they induce fusion between any two or more protoplasts. A more selective and less drastic approach is the electro fusion technique. Studies in the past few years have shown that electric fields can be used for protoplast fusion. This procedure, called electrofusion, has been found to be simpler, quicker and more efficient than chemically induced fusions. More importantly, cells after electrofusion do not show cytotoxic responses as generally found in protoplasts or heterokaryons subjected to PEG treatment. These aspects and the recent demonstrations using electric pulses to introduce foreign DNA into plant cells (electroporation) have further heightened interest in the application of electrofusion in somatic cell genetics.

Senda et al. (1979) first attempted fusion by positioning two microelectrodes with the help of a micromanipulator at the ends of adjoining Rauwolfia protoplasts. Success in inducing fusion was achieved with brief 5-12 amp DC pulses restricted to single protoplast pairs only. Subsequently, Zimmermann and Scheurich (1981) demonstrated that batches of protoplasts could be fused by electric fields by devising a protocol which is now widely used.

This protocol involves a two-step process. First, the protoplasts are introduced into a small fusion chamber containing parallel wires or plates which serve as electrodes. Second, a low-voltage and rapidly oscillating AC field is applied, which causes protoplasts to become aligned into chains of cells (pearl chains) between the electrodes. This creates complete cell-to-cell contact within a few minutes. Once alignment is complete, the fusion is induced by application of a brief spell of highvoltage DC pulses. A high voltage DC pulse induces a reversible breakdown of the plasma membrane at the site of cell contact, leading to fusion and consequent membrane reorganisation. The entire operation is carried out manually in a specially designed equipment, called electroporator, under a microscope. Many workers feel that this fusion technique is more desirable than the others for a number of important reasons. The entire process, starting from the introduction of the protoplasts inside the chamber and their transfer to culture media, can be completed in 5min. or less.

Heterokaryons produced by electrical fusion divide in the culture medium and have demonstrated the capability of regenerating somatic hybrid plants in some cases studied recently. Shoot or somatic hybrid plant regeneration after electrofusion of protoplasts has been reported in combinations: Nicotiana tabacum (+) N. tabacum, N. plumbaginifolia (+) N. tabacum, N. glauca (+) N. langsdorfii, and Solanum tuberosum (+) S. phureja. In addition to these, callus regeneration has been achieved from protoplast combinations of Brassica napus (+) B. napus and Solanum brevidens (+) N. rustica).

Selection of Somatic Hybrids and Cybrids

Somatic Hybrids

Generally, 20-25% protoplasts may be involved in a fusion event although heterokaryon formation as high as 50-100% has been reported. Thus, there is a basic need for selection of the hybrid cells or fusion products. The protoplast suspension recovered after a treatment with a fusion inducing agent (fusogen) consists of the following cell types:

i. unfused protoplasts of the two species/strains,

ii. products of fusion between two or more protoplasts of the same species (homokaryons), and 'hybrid' Protoplasts produced by fusion between one (or more) protoplast(s) of each of the two species (heterokaryons) (Fig.3.). In somatic hybridization experiments, only the heterokaryotic or hybrid protoplasts, particularly those resulting from fusion between one protoplast of each of the two species, are of interest. However, they form only a small proportion of the population (usually 0.5- 10%). Therefore, an effective strategy has to be employed for their identification and isolation. This is called the selection of hybrid cells, and is the most critical, and is still an active area of investigation.

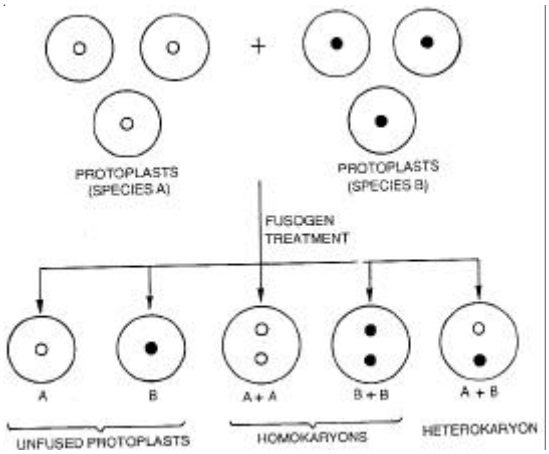

Fig. 3. Different types of products recovered after fusogen (fusion agent) treatment of protoplasts of two different species (A and B) mixed together (usually in 1:1 ratio).

A number of strategies have been used for the selection of hybrid protoplasts:

A. Biochemical Basis for Complementation and Selection

Heterokaryons in fusions involving mesophyll protoplasts from the two parental types cannot be identified and biochemical markers are required allowing only their growth in cultures to form somatic hybrid plants. Carlson et al. (1972) demonstrated the value of a biochemically based selection procedure of somatic hybridisation of Nicotiana species. This selection procedure was based upon a prior knowledge of the nutritional requirements of mesophyll protoplasts isolated from the genetically tumorous Nicotiana glauca and N. langsdorffii. Protoplasts of the hybrid were able to grow in culture to form calli, whereas parental types failed to develop into calli. A truly useful selection system, however, would be one which does not rely upon prior knowledge of the hybrid plants. Other parameters of biochemical complementation in somatic hybrids need to be applied.

i. Drug sensitivity: Power et al. (1976) utilised the differential sensitivity of protoplasts isolated from Petunia parodii and P. hybrida to the drug actinomycin D. In an MS medium, the mesophyll protoplasts of Petunia hybrida develop up to a macroscopic callus stage and those of P. parodii divide to form only small cell colonies. The addition of actinomycin D to the culture medium apparently has little effect on the regeneration potential of parodii protoplasts, but those of P. hybrida fail to divide. Heterokaryons, however, are able to grow despite the presence of the drug and ultimately differentiate into somatic hybrid plants. Asimilar procedure was adopted in the selection of somatic hybrids between Nicotiana sylvestris and N. knightiana.

ii. Auxotrophic mutants: The selection of somatic hybrids as a result of complementation by auxotrophic mutants may be useful, as only the hybrid lines are expected to survive in the minimal medium. Although isolation of such mutants of higher plants is somewhat difficult, Glimelius et al. (1978) succeeded in selection of numerous somatic hybrids by utilizing protoplasts of nitrate reductase-deficient (nitrate non-utilising) and chlorate resistant mutant lines of tobacco isolated by Muller and Grafe. Protoplasts of two genetically different mutants were fused and cultured in a medium containing nitrate as the sole nitrogen source. In control experiments, parental protoplasts did not grow in the presence of nitrate whereas fusion products regenerated. Wallin et al. (1919) also produced somatic hybrids using the same mutants. They fused either normal protoplasts of one mutant with miniprotoplasts of the other mutant or miniprotoplasts of both mutants.

B. Visual Selection

In most of the somatic hybridisation experiments, selection procedures involve fusion of chlorophyll-deficient (non-green) protoplasts of one parent with the green protoplasts of the other parent since this facilitates visual identification of heterokaryons at the light microscope level. Non-green protoplasts are isolated from cultured cells, epidermal cells, or antibiotic-induced albino plantlets (Razdan 1980). Further selection of these heterokaryons to develop somatic hybrid plants in cultures may be achieved by:

i. Complementation selection coupled with differential media growth:

Visual selection procedure is coupled with complementary natural differences in the sensitivity of parental protoplasts to media constituents which enable only the hybrid cells to develop in cultures and regenerate plants. For example, wild type (mesophyll) protoplasts of Petunia parodii fused with albino protoplasts isolated from cell suspension cultures of P. hybrida, P. inflata and P. parviflora in separate experiments. In all these combinations green parodii protoplasts got eliminated at the small colony stage, while the protoplasts of the other parent developed colourless colonies. Hybrid components, contrarily, proliferated into green calli and, subsequently, somatic hybrid plants. Similar procedures were followed in the selection of interspecific somatic hybrids in Daucus, Datura and other genera.

In experiments on intergeneric somatic hybridisation, however, Krumbiegel and Schieder (1979) used the scheme in which the parental protoplasts and heterokaryons were allowed to develop calli in cultures. The morphological differences in the resultant three types of calli permitted the identification of the hybrid tissue, which could then be selected out to regenerate somatic hybrid plants.

ii. Mechanical Isolation:

Individual heterokaryons can be identified visually under a light microscope, isolated mechanically by means of a Drummond pipette and cloned in microdrop cultures (see Lesson13). Gleba and Hoffmann (1979) used this technique for producing somatic hybrid plants: Arabidopsis thaliana (+) Brassica campestris. This approach suffers from the fact that it requires special culture media for each particular hybrid-cell type to divide and form clusters. Hence an alternative has been suggested, namely microdrop culture of

single cells using feeder layers or nursing of heterokaryons by co-culture with phenotypically different protoplasts.

C. Use Of Non-allelic Albino Mutants for Complementation Selection

This selection system was developed by Melchers and Labib in 1974. They fused haploid chlorophyll-deficient and light-sensitive protoplasts of Nicotiana tabacum and cultured them under high intensities of light. After two months green colonies were observed in culture dishes as a consequence of complementation between the two albino mutants. On further culturing, these green colonies regenerated somatic hybrid plants. Non allelic albino mutants have been used successfully to produce intraspecific (Datura innoxia (+) D. innoxia) and interspecific (Petunia parodii (+) P. hy

brida, somtic hybrids.

D. Flow Cytometric Nalysis

Various laboratories are using techniques of flow cytometry and fluorescent-activated cell sorting for the analysis of plant protoplasts whilst maintaining their viability. These techniques have also been applied for the sorting and selective enrichment of heterokaryons. The hybrid calli derived from this sorted material are reported to regenerate hybrid plants in Nicotiana. The procedures established for screening of somatic hybrid plants through fluorescent-activated sorting of fused protoplasts have been comprehensively described by Galbraith (1989).

A more general and widely applicable strategy, but demanding more work than the previous approaches is to culture the entire protoplast population without applying any selection for the hybrid cells. All the types of protoplasts form calli. The hybrid calli are later identified on the basis of callus morphology, chromosome constitution, protein and enzyme banding patterns etc. In some cases, the identification may be delayed till plants are regenerated. In such an approach it will be desirable to culture the protoplasts in very low densities since neighbouring colonies are likely to fuse at higher densities. Ideally, they should be cultured in microdrops, each drop containing but a single cell. Many workers tend to favour this approach since it does not depend on the presence of appropriate but difficult to find markers in the parental species.

Regeneration of Hybrid Plants

Once hybrid calli are obtained, plants are induced to regenerate from them since this is a prerequisite for their exploitation in crop improvement. Further, the hybrid plants must be at least partially fertile, in addition to having some useful property, to be of any use in breeding schemes. The culture techniques have been refined to a state where plant regeneration has been obtained in a number of somatic hybrids (Table.1.). But even today, it has not been possible to recover hybrid plants and/or calli from a number of somatic combinations; this phenomenon is called 'somatic incompatibility'. The reasons for somatic incompatibility are not clearly understood.

Symmetric Hybrids. Some somatic hybrid plants retain the full or nearly full somatic complements of the two parental species; these are called symmetric hybrids. Such hybrids provide unique opportunities for synthesizing novel species which may be of theoretical and/or practical interest.

Frequently, somatic hybrids (symmetric) between distantly related sexually incom-patible species are sterile, precluding their incorporation in a breeding programme. This may be circumvented by producing 3n somatic hybrids by fusing somatic (2n) cells of one species with haploid (n) cells of the other species; such 3n plants may be expected to be partially fertile. These somatic hybrids can now be used in breeding programmes for limited gene/chromosome introgression from the species contributing the haploid protoplast.

TABLE 1. A list of some distant somatic hybrid plants.

Symmetric or near-symmetric Hybrids
Salanun tuberosum + Lycopersicon esculentum*
Datura innoxia + Atropa belladona
Arabidopsis thaliana + Brassica campestris
Atropa belladona + Nicotiana chinensis
Asymmetric Hybrids
Daucus carota + Aegopodium podagraria
Daucus carota + Petroselinum hortense
Hyoscyamus muticus + Nicotiana tabacum
Datura innoxia + Physalis minima
Nicotiana tabacum + Daucus carota

* The + symbol indicates that the hybrid was obtained through protoplast fusion.

An approach to the improvement of apparently useless somatic hybrids, e.g., nonflowering somatic hybrid Daucus carota + Aegopodium podagrarira, is to fuse protoplasts from the hybrid with those of one of the parental species. The fusion of a somatic hybrid protoplast with that from one of its parents is called somatic back hybridization. When protoplasts from the above somatic hybrid were fused with carrot protoplasts, the resulting somatic hybrid produced flowers. Such hybrids can now be ordered into breeding programmes with the aim of gene/chromosome introgression.

Asymmetric Hybrids. Many somatic hybrids exhibit the full somatic complement of one parental species, while all or nearly all chromosomes of the other parental species are lost during the preceding mitotic divisions; such hybrids are referred to as asymmetric hybrids. The available evidence suggests that such hybrids are likely to show a limited introgression of chromosome segments from the eliminated genome(s) due to drastically enhanced chromosomal aberrations and/or mitotic crossing over in vitro. Asymmetric hybrids can be obtained even from those combinations which normally produce symmetric hybrids by the following approach:

Protoplasts of one of the parental species are irradiated with a suitable dose of X-rays or gamma-rays to induce extensive chromosome breakage. In such cases, chromosome segment introgression may be markedly enhanced. It may be pointed out that asymmetric hybrids are essentially cytoplasmic hybrids or cybrids except for the introgressed genes.

Fate of Plasma Genes. In contrast to sexual hybrid cells, i.e., zygotes, which contain the cytoplasmic genes (plasmon) from the female parent only, somatic hybrid cells contain cytoplasmic complements from both the parental species. The cytoplasmic

genes (generally studied in terms of chloroplast types or chloroplast DNA, cp-DNA) appear to be distributed randomly during the mitotic cell divisions. As a result, some cells receive chloroplasts of one parental species, some others of the other species and a small proportion retain the chloroplasts of both the species. This is reflected in the plants regenerated from these cells. The same applies to mitochondria as well. In addition, the distribution of chloroplasts is independent from that of mitochondria. Therefore, a somatic hybrid plant may contain chloroplasts from one parental species and mitochondria from the other fusion parent. There is considerable evidence that the genomes of both chloroplasts and mitochondria, particularly the latter, undergo recombination in the hybrid cells; this produces recombinant organelles in the progeny.

Cybrids

What are cybrids and how are they produced?

Cybrids or cytoplasmic hybrids are cells or plants containing nucleus of one species but cytoplasm from both the parental species. They are produced in variable frequencies in normal protoplast fusion experiments due to one of the following:

i. fusion of a normal protoplast of one species with an enucleate protoplast or a protoplast having an inactivated nucleus of the other species,

ii. elimination of the nucleus of one species from a normal heterokaryon, or

iii. gradual elimination of the chromosomes of one species from a hybrid cell during the subsequent mitotic divisions. Cybrids may be produced in relatively high frequency by (i) irradiating (with X-rays or gamma-rays) the protoplasts of one species prior to fusion in order to inactivate their nuclei, or (ii) by preparing enucleate protoplasts (cytoplasts) of one species and fusing them with normal protoplasts of the other species.

The objective of cybrid production is to combine the cytoplasmic genes of one species with the nuclear and cytoplasmic genes of another species. But the mitotic segregation of plasma genes, as evidenced by the distribution of chloroplasts, leads to the recovery of plants having plasma genes of one or the other species only. Only a small proportion of the plants remain 'cybrid' which would further segregate into the two parental types.

This provides the following unique opportunities:

i. transfer of plasma genes of one species into the nuclear background of another species in a single generation and even in

ii. sexually incompatible combinations,

iii. recovery of recombinants between the parental mitochondrial or chloroplast DNAs (genomes), and

iv. production of a wide variety of combinations of the parental and. recombinant chloroplasts with the parental or recombinant mitochondria. When cybrids are produced by irradiating the protoplasts of one species prior to fusion, they provide the additional opportunity for

v. recovery of chromosome segment introgressions from the lost genome, in combination with variations in the plasmon. The cybrid approach has been used for the transfer of cytoplasmic male sterility from Nicotiana tabacum to N. sylvestris, from Petunia hybrida to P. axillaris etc.

vi. In addition, mitochondria from one parental species may be combined with the chloroplasts of the other parental species.

Practical Applications of Somatic Hybridisation and Cybridisation

1. Means of Genetic Recombination in Asexual or Sterile Plants

Somatic cell fusion appears to be the only approach through which two different parental genomes can be recombined among plants that cannot reproduce sexually. Further, protoplasts of sexually sterile (haploid, triploid and aneuploid) plants can be fused to produce fertile diploids and polyploids. There are several reports describing the amphidiploid and hexaploid plants produced from fusion of haploid protoplasts of tobacco. Protoplasts isolated from dihaploid potato clones have been fused with isolated protoplasts of Solanum brevidens to produce hybrids of practical breeding value (Fish et al. 1988). Haploid protoplasts from an anther-derived callus of rice cultivars, upon fusion also produce fertile diploid and triploid hybrids (Toryama and Hinata 1988).

2. Overcoming Barriers of Sexual incompatibility

In plant breeding programmes, sexual crossings at interspecific or intergeneric levels often fail to produce hybrids due to incompatibility barriers. The bottlenecks in sexual hybridisation may therefore, be overcome by somatic cell fusion. In some cases somatic hybrids between two incompatible plants have also found application in industry or agriculture.

Schieder (1978) obtained amphidiploid Datura innoxia (+) D. discolor and D. innoxia (+) D. stramonium, by fusing their diploid mesophyll protoplasts. These hybrids did not exist in nature as conventional breeding procedures proved unsuccessful. Somatically produced amphidiploids of these combinations of Datura species are propagated for industrial uses as they demonstrate heterosis and higher (20-25%) scopolamine content than in the parental forms.

Nicotiana repanda, N. nesophila and N. stockonii are resistant to a number of diseases but are not sexually crossable with tobacco (N. tabacum). However, fertile hybrids have been reported in combination N. tabacum (+) N. nesophila and N. tabacum (+) N. stocktonii by protoplast fusion. Somatic hybridisation of dihaploid and tetraploid potato protoplasts with isolated protoplasts of Solanum brevidens, S. phureja and S. pennel/ii resulted in the synthesis of fertile, partially amphieuploid plants possessing important agricultural traits, e.g., resistance to potato leaf virus, potato virus Y and Erwinia soft rot. Using this approach, tomato (Lycopersicon esculentum) hybridised somatically with a number of wild species has resulted in the synthesis of hybrids which are fertile and used in breeding programmes. Interspecific somatic hybridisation involving species that are sexually incompatible with egg-plant (Solanum melongena) has also resulted in the production of amphidiploids with traits resistant to verticillium wilt (Guri and Sink 1988).

Rapeseed (Brassica napus) is a natural amphidiploid of B. o/eraeea and B. campestris. Schenk (1982) was the first to resynthesise rapeseed in vitro using protoplast fusion. Somatic hybridisation between B. napus and B. nigra cultivar, possessing the gene for resistance to Phoma lingam, yielded amphidiploid plants carrying this gene. These hybrids possess all the three Brassica genomes (A, Band C) and are now incorporated in breeding programmes (Sjodin and Glimelius 1989a, b). Recently, hybrids have been produced parasexually by protoplast fusion, between Brassica juncea (a major oilseed crop of the tropical world) and the sexually incompatible species Diplotaxis muralis (Chatterjee et al. 1988) and Erica sativa (Sikdar et al. 1990).

The potential of somatic hybridisation in perennial tree breeding is best illustrated by interspecific and intergeneric somatic hybridisation among citrus species. Somatic hybrids produced through these experiments are amphidiploids featuring characteristics for scion improvement and increased rootstock potential. Other combinations resulting in the synthesis of somatic hybrids and cybrids are summarised in Appendix 12.2.

Cytoplasm Transfer

Power et al. (1975) fused mesophyll protoplasts of Petunia with cultured cell protoplasts of the crown gall of Parthenocissus and selected a line which contained the chromosomes of only Parthenocissus but exhibited some of the cytoplasmic properties of Petunia for sometime. This was followed by direct application of cybridisation in agricultural biotechnology by transfer of cytoplasmic male sterility from Nicotiana techne to N. tabacum (Belliard et al. 1978), N. tabacum to N. sylvestris. (Zelcer et al. 1978, Aviv et al. 1980) and Petunia hybrida to P. axillaris (Izhar and Power 1979). Besides cytoplasmic male sterility, the genophore of the cytoplasm codes for a number of practically important traits, such as the rate of photosynthesis, low or high temperature tolerance and resistance to diseases or herbicides. Recent experiments on cybridisation have resulted in plants with reconstructed cytoplasm combining mitochondrial DNA (mt DNA) and cp DNA encoded traits from both parents.

The best example illustrating the potential for protoplast fusion in reconstructing cytoplasm for practical purposes is the genus Brassica. Two desirable traits coded by cytoplasmic genes have been genetically manipulated through interspecific cybridisation between different species of Brassica. These traits include cytoplasmic male sterility (cms) and resistance to atrazine herbicides. The cms gene in Brassica plants, Diplotaxis muralis and Raphanus sativus is of alloplasmic (the nucleus of one species into a foreign cytoplasm) origin. Raphanus sativus is of interest because it leads to complete male sterility. Cms restorer genes have been introduced into rapeseed (Brassica napus) from this plant. Mutants resistant to atrazine herbicide have also been discovered both in Brassica napus and B. campesteris. Protoplast fusion experiments (conducted in various laboratories) have resulted in the synthesis of cybrid plants with reconstructed cytoplasm combining both cms (coded by Raphanus mt DNA) and low temperature tolerance or atrazine resistance (coded by Brassica cp DNA). Similarly, cytoplasmic genes coding for atrazine resistance and cms have been transferred into cabbage, rice and potato (see Bajaj 1989b, Gleba and Shlumukov 1990).

Present Perspective

In somatic hybridisation and cybridisation, the essential pre requisite is that parental protoplasts and their fusion products regenerate to whole plants. Research in the past decade has shown that plants can be raised in vitro from isolated protoplasts of species belonging to a range of angiosperm families. Somatic hybrids have been produced between sexually compatible as well as incompatible species. It could be possible to overcome prezygotic embryo/endosperm (Petunia parodii (+) P. inflata (Power et al. 1979) and postzygotic (Datura innoxia (+) D. stramonium, Schieder 1978; Petunia parodii (+) P. parviflora, Power et al. 1980) incompatibility barriers by protoplast fusion. Experiments on intergeneric somatic hybridisation have also been successful in some cases such as potato (+) tomato somatic hybrids (Melchers et al. 1978) and synthesis of 'Arabidobrassica' (Gleba and Hoffmann, 1979). With these initial successes, and subsequent advancements in protoplast technology it is desirable that efforts be concentrated on important plant species which have potential in industry or for food production. Crops which have not yielded satisfactory results through conventional methods of genetic manipulation need to be aided by non-conventional in vitro techniques such as somatic hybridisation/cybridisation, embryo culture, etc. to manifest their full potential.

Conclusion

This calls for a 'broad spectrum' approach for the genetic improvement of crops. Even somatic hybrids of sexually compatible plants may exhibit new variations as a result of interactions between plastomes donated by parental species during protoplast fusion. The technique of cybridisation, besides transfer of male sterility, can be adopted for the introduction of genes for resistance into the new species. The modification of plants with respect to nitrogen fixation can also be contemplated through transformation of protoplasts by uptake of exogenous DNA, or organelles, carrying this trait. Further, genetically heterogenous clones can be derived from protoplast culture and fusion which display a high frequency of variations for several agronomic traits.

The above developments suggest an immense potential for somatic cell genetics in crop improvement. However, the genetic diversity that can be generated via somatic cell fusion is still poorly understood. This is because only a very limited number of the synthesised somatic hybrids or cybrids have been fertile or amphiploids. Induction and control over the degree of species-specific chromosome elimination in wide or distant somatic hybridisation requires to be mastered in order to understand the mechanism of producing desirable asymmetric nuclear hybrids.

Questions

1. Give a brief account of the different fusion strategies followed for protoplast fusion.

2. What is somatic hybridization? Briefly describe the various steps in somatic hybridization with special emphasis on selection of hybrid cells.
3. Write short notes on:
   a. Recovery of hybrid plants
   b. Cybrids.

Note